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Sensitive determination of omeprazole and its two main metabolites in human plasma by column-switching high-performance liquid chromatography: Application to pharmacokinetic study in relation to CYP2C19 genotypes

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Abstract

A simple and sensitive column-switching high-performance liquid chromatographic method was developed for the simultaneous determination of omeprazole and its two main metabolites, 5-hydroxyomeprazole and omeprazole sulfone, in human plasma. Omeprazole, its two metabolites and lansoprazol as an internal standard were extracted from 1 ml of alkalinized plasma sample using diethyl ether–dichloromethane (45:55, v/v). The extract was injected into a column I (TSK-PW precolumn, 10 μm, 35 mm × 4.6 mm i.d.) for clean-up and column II (Inertsil ODS-80A column, 5 μm, 150 mm × 4.6 mm i.d.) for separation. The mobile phase consisted of phosphate buffer–acetonitrile (92:8 v/v, pH 7.0) for clean-up and phosphate buffer–acetonitrile–methanol (65:30:5 v/v/v, pH 6.5) for separation, respectively. The peak was detected with an ultraviolet detector set at a wavelength of 302 nm, and total time for chromatographic separation was ∼25 min. The validated concentration ranges of this method were 3–2000 ng/ml for omeprazole, 3–500 ng/ml for 5-hydroxyomeprazole and 3–1000 ng/ml for omeprazole sulfone. Mean recoveries were 84.3% for omeprazole, 64.3% for 5-hydroxyomeprazole and 86.1% for omeprazole sulfone. Intra- and inter-day coefficient variations were less than 5.1 and 6.6% for omeprazole, 4.6 and 5.0% for 5-hydroxyomeprazole and 4.6 and 4.9% for omeprazole sulfone at the different concentrations. The limits of quantification were 3 ng/ml for omeprazole and its metabolites. This method was suitable for use in pharmacokinetic studies in human volunteers, and provides a useful tool for measuring CYP2C19 activity.

Keywords: Omeprazole; 5-Hydroxyomeprazole; Omeprazole sulfone; CYP2C19; HPLC

1. Introduction

Omeprazole (5-methoxy-2-{[(4-methoxy-3,5-dimethyl-2-pyridinyl) methyl] sulfinyl}-1H-benzimidazole) is a selective inhibitor of gastric acid secretion by interacting with H^+/K^+-ATPase in gastric parietal cells [1]. Clinical trials have shown that omeprazole was highly effective for the treatment of duodenal or gastric ulcer, reflux esophagitis and Zollinger–Ellison syndrome [2,3]. Recently, a triple therapy regimen with omeprazole, amoxicillin and clarithromycin is also widely accepted for *Helicobacter pylori* (*H. pylori*) eradication [4–6].

Omeprazole is extensively metabolized to the primary metabolite of 5-hydroxyomeprazole and omeprazole slufone. The formation of 5-hydroxyomeprazole is mainly mediated by cytochrome P450 (CYP) 2C19, whereas the formation of omeprazole sulfone is by CYP3A4 (Fig. 1) [7,8]. The pharmacokinetics and pharmacodynamics of omeprazole significantly depend on *CYP2C19* phenotype or genotype status. The area under the plasma-concentration–time curve (AUC) of oral and intravenous omeprazole in poor metabolizers (PMs) of CYP2C19 is 6- to 10-fold larger than that of extensive metabolizers (EMs) [9–11]. The higher plasma concentration of

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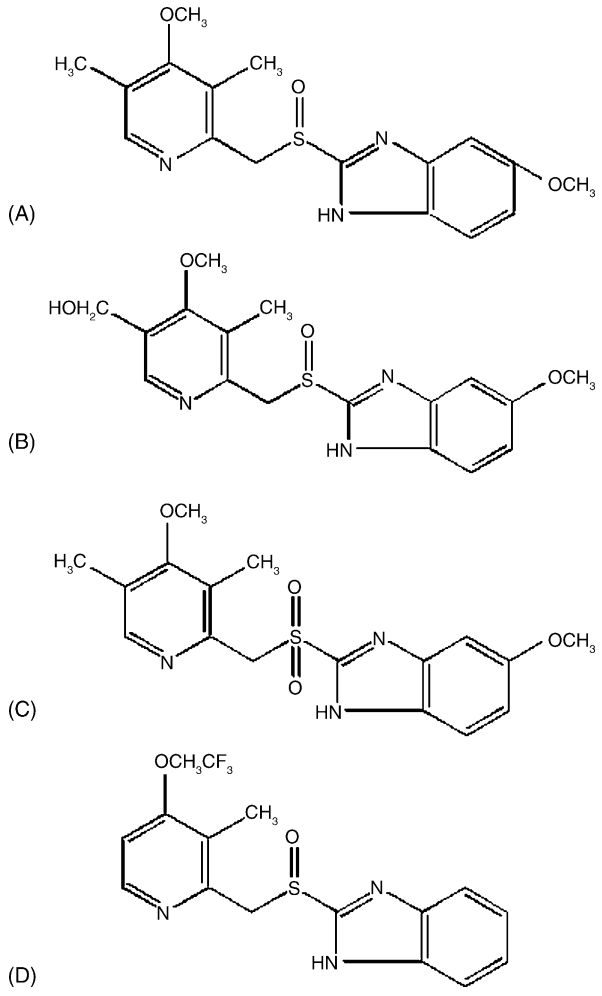

Fig. 1. Chemical structures of (A) omeprazole, (B) 5-hydroxyomeprazole, (C) omeprazole sulfone and (D) lansoprazole as an internal standard.

omeprazole in the PMs showed higher effectiveness in anti-*H. pylori* therapy, higher gastric pH, and possibly higher stability of antimicrobials [12]. Omeprazole hydroxylation index (the AUC of omeprazole/the AUC of 5-hydroxyomeprazole) has been used as a marker of CYP2C19 activity [13,14], whereas, the sufoxidation index (the AUC of omeprazole/the AUC of omeprazole sulfone) has been used to evaluate the activity of CYP3A4 [15,16]. Therefore, a reliable analysis of omeprazole and its metabolites is essential to evaluate the indexes of CYP2C19 or CYP3A4 in the clinical situation.

Several HPLC methods including enantioselective or mass spectrometric detection have been reported for the determination of omeprazole in human plasma [17–24]. However, a few publications have reported a simple HPLC method for the simultaneous determination of omeprazole, 5-hydroxyomeprazole and omeprazole sulfone in human plasma [22–24], and there is no information describing the simultaneous determination of omeprazole and its main metabolites concentrations by a column-switching HPLC method after a liquid-liquid extraction. However, we have reported serial analytical methods for proton-pump inhibitors, lansoprazole and rabeprazole, by using column-switching HPLC method [25,26].

In addition, although previous methods have used a mobile phase with relatively high pH values of ≥8.0 [22–24], mobile phases with pH values of 8 or more tend to shorten the life of common reversed phase columns [27]. Kobayashi et al. [23] have reported a simple liquid–liquid extraction procedure for omeprazole and its two main metabolites concentrations with a mobile phase of pH 8.5, and achieved low quantification limit (10 ng/ml) for omeprazole, 5-hydroxyomeprazole and omeprazole sulfone. However, high pH values in the method needed a more expensive alkaline-resistant column but not a standard silica based column to prevent decomposition of compounds. Gonzalez et al. [23] achieved a simultaneous determination of omeprazole and its two metabolites by using a standard ODS C18 column with the mobile phase of pH 8.0 containing 0.015% nonylamine, and this prolonged the life of the column for analysis. However, the high quantification limits (60 ng/ml) of three compounds in that method are not sufficiently sensitive to obtain precise pharmacokinetic parameters. Generally, a large inter-individual variation that depended on *CYP2C19* genotype was observed in the pharmacokinetics of omeprazole. In addition, several in vivo studies have consistently shown that 5-hydroxyomeprazole concentration of PMs is much less than that of EMs, and omeprazole sulfone concentration of PMs is much greater than that of EMs [14,28]. Therefore, a more sensitive method having a wider range of capability is required for evaluating the precise pharmacokinetics of omeprazole and its metabolites in relation to *CYP2C19* genotypes. In the present study, we describe a new and sensitive column-switching HPLC method for the simultaneous determination of omeprazole and its metabolites, 5-hydroxyomeprazole and omeprazole sulfone in human plasma using a liquid–liquid extraction. The assay is suitable for pharmacokinetics study in relation to *CYP2C19* genotype status.

2. Experimental

2.1. Chemicals and reagents

Omeprazole (purity 99.0%) and its metabolites, 5-hydroxyomeprazole (purity 99.8%) and omeprazole sulfone (purity 99.5%) were kindly provided by Astra Zeneca R & D (Molndal, Sweden), and lansoprazole (purity 99.4%) as an internal standard (I.S.) was kindly provided by Takeda Chemical Industries (Osaka, Japan) (Fig. 1). Disodium hydrogen phosphate, acetonitrile, methanol, diethyl ether and dichloromethane were purchased from Wako Pure Chemical Industries (Osaka, Japan). Water was deionized and purified using a Milli-Q system (MP-650, IWAKI Millipore, Tokyo, Japan).

2.2. Preparation of stock and working solutions

Stock solutions of omeprazole, 5-hydroxyomeprazole, omeprazole sulfone and I.S. were prepared by dissolving an appropriate amount of each compound in methanol to yield concentrations of 1.0 mg/ml for generating standard curves. Working standard solutions of omeprazole, 5-hydroxyomeprazole, omeprazole sulfone (100, 10, 1 and 0.1 μg/ml) were prepared by serial dilution with methanol. The working standard solutions

of I.S. (20 μg/ml) was obtained by 50 times diluting the stock solution (1.0 mg/ml) with 50 mM disodium hydrogen phosphate. Stock solutions were stable at −20 °C at least 6 months for the analysis.

2.3. Extraction procedure

I.S. (lansoprazole) 20 μl of 20 μg/ml and 0.1 ml of 0.5 M disodium hydrogen phosphate were added to 1 ml of plasma. The tubes were vortex-mixed for 10 s and 4 ml of diethyl ether–dichloromethane (45:55, v/v) was added as extraction solvent. After 10 min of vortex-mixing, the mixture was centrifuged at 2500 × *g* for 10 min at 4 °C (KUBOTA, 5910, Kubota, Tokyo, Japan), and the organic phase (3.5 ml) was evaporated in vacuo at 50 °C to dryness (TAITEC VC-960, Shimadzu, Kyoto, Japan). The residue was dissolved with 30 μl of methanol and 100 μl of 50 mM disodium hydrogen phosphate buffer (pH 9.3), 30 μl aliquot was injected onto the column.

2.4. Instrumentation and chromatographic condition

The column-switching HPLC system consisted of two Shimadzu LC-10AT high-pressure pumps (for eluent A and B), a Shimadzu CTO-10A column oven, a Shimadzu Work station CLASS-VP chromatography integrator (Kyoto, Japan), a Shimadzu SPD-10AVP (Kyoto, Japan) and a Shimadzu autosampler SIL-10ADVP (500 μl injection volume) (Kyoto, Japan). A TSK gel PW precolumn (a hydrophilic metaacrylate polymer column) for sample clean-up (column I; 35 mm × 4.6 mm i.d., particle size 10 μm; Tosoh, Tokyo, Japan) and an Inertsil ODS-80A column as an analytical column (column II; 150 mm × 4.6 mm i.d., particle size 5 μm; GL Science Inc, Tokyo, Japan) were used.

Column-switching chromatographic condition was set based on our previous report relevant to other PPIs, rabeprazole and lansoprazole [25,26]. A 30-μl portion of the extract was automatically injected into the HPLC system. The column-switching system and flow-rates were operated according to the time program depicted in Table 1. The mobile phases consisted of phosphate buffer–acetonitrile (eluent A) (92:8 v/v, pH 7.0) for clean-up and phosphate buffer–acetonitrile–methanol (eluent B) (65:30:5 v/v/v, pH 6.5) for separation, respectively. The temperatures of columns were maintained at 40 °C. The peak was detected by an ultraviolet detector set at a wavelength of 302 nm. The peak area was used for the quantification of omeprazole, 5-hydroxyomeprazole and omeprazole sulfone.

2.5. Pharmacokinetic study design and sample collections

Eleven subjects (9 men and 2 women)underwent a CYP2C19 genotyping test by use of a polymerase chain reaction–restriction fragment length polymorphism method with allele-specific primer for identifying the CYP2C19 wild-type (*1) gene and the 2 mutated alleles, CYP2C19*2 (*2) in exon 5 and CYP2C19*3 (*3) in exon 4 [29]. On the basis of the results, subjects were classified into 1 of 3 genotype groups as follows: homozygous EMs (*1/*1, 3 subjects), heterozygous EMs (*1/*2 or *1/*3, 4 subjects) or PMs (*2/*2 or *2/*3, 4 subjects). The Ethics Committee of Hirosaki University School of Medicine approved this study protocol, and written informed consent had been obtained from each participant before any examinations. This study was conducted in cross over-randomized manner with at least a 2-week interval between testing. They took either 20 mg omeprazole (Omepral® Injection 20, AstraZeneca Co., Osaka, Japan) by intravenous injection (IV) for 1 min or 40 mg omeprazole (two tablets of Omepral®, AstraZeneca Co., Osaka, Japan) with water (PO). Blood samples (10 ml) were obtained before and 0.08, 0.5, 1, 2, 3, 4, 6 and 8 h after the IV dosing, and before and 0.5, 1, 1.5, 2, 3, 4, 6 and 8 h after the PO dosing. Plasma samples were frozen and kept at −20 °C until analysis.

2.6. Pharmacokinetic data analysis and statistical analyses

The maximum plasma concentration (C_{max}) of omeprazole and its metabolites and the time to reach C_{max} (t_{max}) were determined directly from the individual concentration–time data. Pharmacokinetic parameters were calculated by non-compartmental methods. The terminal elimination rate constant (k_e) was obtained by linear regression analysis by use of at least three sampling points of the terminal log–linear declining phase to the last measurable concentration. The apparent elimination half-life ($t_{1/2}$) was calculated as 0.693 divided by k_e. The area under the plasma concentration–time curve from time zero to the last sampling time (AUC_{0-t}) was calculated by the trapezoidal rule. The hydroxylation metabolic index was calculated as AUC_{0-8} of omeprazole/AUC_{0-8} of 5-hydroxyomeprazole. The sulfoxidation metabolic index was calculated as AUC_{0-8} of omeprazole/AUC_{0-8} of omeprazole sulfone.

The pharmacokinetic parameters of omeprazole and its metabolites among three different genotype groups were compared using one-way ANOVA, and Scheffe's test was used for post hoc comparison. p-values of < 0.05 was considered statistically significant for all tests.

Table 1
Time program for the column switching HPLC

Time after injection (min)	Column I		Column II	
	Mobile phase	Flow rate (ml/min)	Mobile phase	Flow rate (ml/min)
0.0–7.0	A	1.2	B	0.8
7.0–10.0	B	0.8	B	0.8
10.0–17.0	A	1.2	B	0.8
17.0–25.0	A	1.2	B	1.5

Mobile phase A: pH 7.0 0.05 M Na_2HPO_4 92: AcCN 8. Mobile phase B: pH 6.5 0.05 M Na_2HPO_4 65: AcCN 30: MeOH 5.

3. Results and discussion

3.1. Chromatographic optimization

This paper describes a new method for the simultaneous determination of omeprazole, 5-hydroxyomeprazole and omeprazole sulfone in human plasma by column-switching HPLC. Initially, our goal was to develop a more sensitive method to obtain precise pharmacokinetics of omeprazole and its two metabolites than had previously been reported. In previous reports, a liquid–liquid extraction with dichloromethane was used for the sample preparation of omeprazole and/or its metabolites [20,21,23]. However, our study required a different extractable solvent because interfering peaks presented at the same times of 5-hydroxyomeprazole and omeprazole sulfone when dichloromethane was used. Therefore, we used diethyl ether–dichloromethane (45:55, v/v) as an extractable solvent, and then an automated column-switching system for plasma clean-up because only a liquid–liquid extraction showed the LOQ of 5-hydroxyomeprazole was lower sensitive for more significant blank response. Additionally, our extraction method enabled the determination of all plasma samples by one analytical column. Consequently, there were no interfering peaks of endogenous substances with a retention time similar to the peaks of omeprazole, 5-hydroxyomeprazole and omeprazole sulfone (Fig. 2), and the limit of quantification of 3 ng/ml for each compound in this study was more sensitive than that of previous methods [22–24].

Additionally, we achieved a successful resolution of these compounds and I.S. by lowering the pH of the mobile phases (eluent A, pH 7.0; eluent B, pH 6.5). In previous reports [22–24], the simultaneous determination of omeprazole and its metabolites had used a mobile phase with relatively high pH values of ≥8.0 because of the instability of omeprazole under acidic condition [30,31] and a better resolution omeprazole and omeprazole sulfone [32]. However, a mobile phase with high pH values was not used for the standard HPLC analysis because it could cause a rapid deterioration of normally used silica based columns. In our preliminary study, when we used eluent A of pH 7.0 or eluent B of pH 6.5 for the residues, omeprazole, 5-hydroxyomeprazole, omeprazole sulfone and I.S. in extracts were constant numbers of the peak areas at room temperature for 24 h in the autosampler. This result indicated that lowering the pH of our mobile phases would not decompose omeprazole and its metabolites during the short run-time (within 25 min) for chromatographic separation. This method is a little time consuming. In addition, our precision and accuracy data at the different concentration ranges was validated according to FDA Guideline [33] (Table 3). Therefore, this study is the first report enabling the simultaneous determination of omeprazole and its metabolites with a mobile phase of <8.0.

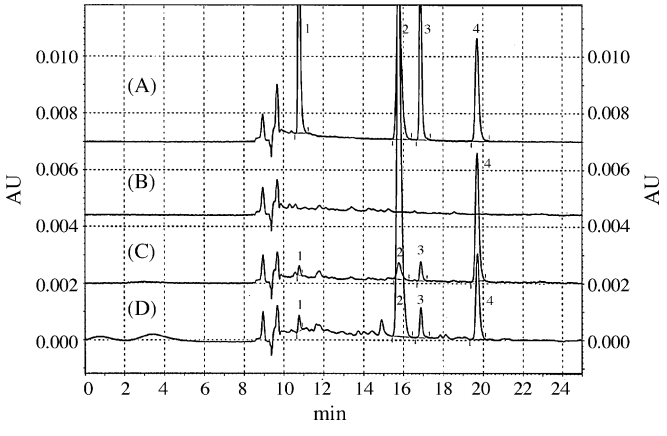

Fig. 2. Typical chromatograms of (A) working solution, (B) blank plasma, (C) plasma spiked with 5 ng/ml of 5-hydroxyomeprazole, omeprazole, omeprazole sulfone and I.S., and (D) plasma obtained from one volunteer of PMs at 1.5 h after an oral administration of omeprazole (40 mg); the plasma concentrations of 5-hydroxyomeprazole, omeprazole, and omeprazole sulfone were 23.5, 1560.5 and 20.8 ng/ml. Peaks: (1) 5-hydroxyomeprazole, (2) omeprazole, (3) omeprazole sulfone and (4) I.S.

3.2. Linearity

Calibration curves were linear over the concentration range from 3 to 2000 ng/ml ($r^2 = 0.9999$ and $F = 141475.4$, $p < 0.001$, $n = 5$) for omeprazole, from 3 to 500 ng/ml ($r^2 = 0.9997$ and $F = 27541.9$, $p < 0.001$, $n = 5$) for 5-hydroxyomeprazole, and from 3 to 1000 ng/ml ($r^2 = 0.9999$ and $F = 38390.7$, $p < 0.001$, $n = 5$) for omeprazole sulfone (Table 2).

3.3. Specificity and sensitivity

A typical chromatogram of working solution is shown in Fig. 2A; the retention times of 5-hydroxyomeprazole, omeprazole, omeprazole sulfone and I.S. were 10.8, 15.8, 16.9 and 19.7 min, respectively. A typical chromatogram of an extracted blank plasma sample is shown in Fig. 2B, while the chromatogram of an extracted sample spiked with 5 ng/ml of omeprazole, 5-hydroxyomeprazole, omeprazole sulfone and I.S., is shown in Fig. 2C. The chromatogram of extracted plasma sample obtained from one volunteer at 1.5 h after receiving 40 mg omeprazole did not show interference peaks (Fig. 2D).

The limit of detection was defined as analyte responses was at least five times greater than the response as compared to blank response (signal-to-noise ratio = 5), and 1.5 ng/ml for omeprazole, 5-hydroxyomeprazole and omeprazole sulfone, respectively. The lowest standard on the calibration curve was defined as the limit of quantification by which the analyte peaks for three compounds were identifiable, discrete and reproducible with a precision of 20% and accuracy of 80–120%. The limits of quantification were 3 ng/ml for omeprazole, 5-hydroxyomeprazole and omeprazole sulfone, respectively.

Potential interference from co-administrated drugs was investigated by determining their retention times in this system. In plasma samples from a subjects taking clarithromycin and amoxicillin given for *H. pylori* eradicative therapy, no peaks were observed to interfere the peaks of omeprazole, 5-hydroxyomeprazole, omeprazole sulfone and I.S.

3.4. Recovery (extraction efficiency) from matrix

Recovery from plasma was calculated by comparing the peak areas of pure standards prepared in working solution,

Table 2
Individual and mean values for slope, intercepts, correlation coefficients and F-values of five calibration curves for analytes

Analyte	Curve	Slope	Intercepts	r^2	F-test for lack of linearity		Concentration added (ng/ml)	Found (mean ± S.D.)	Accuracy (%)	C.V. (%)	n
					F	p-value					
Omeprazole	1	0.0153	0.1274	0.9997	12805.3	<0.001	3	2.94 ± 0.12	98.12	4.16	5
	2	0.0148	0.0761	1.0000	95201.0	<0.001	10	9.87 ± 0.56	98.72	5.68	5
	3	0.0137	0.0664	0.9998	24728.2	<0.001	50	47.29 ± 2.44	94.58	5.17	5
	4	0.0152	0.1505	0.9999	22702.6	<0.001	200	201.62 ± 3.98	100.81	1.97	5
	5	0.0136	0.0228	1.0000	551940.2	<0.001	500	502.12 ± 8.19	100.42	1.63	5
							1000	995.42 ± 3.62	99.54	0.36	5
							2000	1939.51 ± 58.70	97.66	3.01	5
	Mean	0.0145	0.0886								
	S.D.	0.0008	0.0508								
	S.E.	0.0004	0.0227								
5-Hydroxyomeprazole	1	0.0100	0.0325	0.9999	54443.3	<0.001	3	2.89 ± 0.14	96.29	4.88	5
	2	0.0131	0.0337	0.9998	25276.5	<0.001	5	4.87 ± 0.24	97.32	5.03	5
	3	0.0127	0.0102	0.9998	23845.5	<0.001	25	25.03 ± 2.04	100.13	8.17	5
	4	0.0152	−0.0442	0.9994	6602.2	<0.001	100	99.24 ± 5.25	99.24	5.29	5
	5	0.0119	0.0398	0.9999	20472.7	<0.001	250	252.29 ± 3.86	100.91	1.53	5
							500	497.46 ± 1.93	99.49	0.39	5
	Mean	0.0126	0.0144								
	S.D.	0.0019	0.0346								
	S.E.	0.0008	0.0155								
Omeprazole sulfone	1	0.0078	0.1280	0.9998	19253.4	<0.001	3	2.90 ± 0.17	96.68	5.96	5
	2	0.0073	0.0351	0.9999	30325.4	<0.001	10	9.87 ± 0.26	98.70	2.68	5
	3	0.0068	0.0684	1.0000	81597.7	<0.001	50	47.54 ± 3.14	95.08	6.60	5
	4	0.0076	0.0364	0.9999	22386.1	<0.001	200	205.46 ± 3.37	102.73	1.64	5
	5	0.0069	0.0035	0.9999	47157.8	<0.001	500	500.00 ± 6.03	100.00	1.21	5
							1000	998.70 ± 6.30	99.87	0.63	5
	Mean	0.0073	0.0543								
	S.D.	0.0004	0.0472								
	S.E.	0.0002	0.0211								

and injected directly into the analytical column with those of extracted plasma samples containing the same amount of the test compounds ($n = 6$). Mean absolute recoveries were 82.8–86.4% for omeprazole at 4, 900 and 1600 ng/ml, 62.3–66.8% for 5-hydroxyomeprazole at 4, 250 and 400 ng/ml, and 85.1–87.0% for omeprazole sulfone at 4, 400 and 800 ng/ml, respectively, and their mean CV values were 4.4, 7.0 and 5.0%, respectively. The mean recovery for internal standard was 70.6%.

3.5. Precision and accuracy

Intra- and inter-day precision and accuracy were evaluated by assaying quality controls with different concentrations

Table 3
Presicion and accuracy for determination of analytes in spiked plasma ($n = 6$)

Analyte	Concentration added (ng/ml)	Found (mean ± S.D.) (ng/ml)	Accuracy (%)	Intra-day		Found (mean ± S.D.) (ng/ml)	Accuracy (%)	Inter-day	
				C.V. (%)	Relative error (%)			C.V. (%)	Relative error (%)
Omeprazole	4	3.88 ± 0.15	97.23	4.01	−2.77	3.83 ± 0.08	95.86	1.99	−4.14
	80	79.75 ± 2.16	99.69	2.70	−0.31	80.06 ± 2.25	102.51	2.75	1.64
	900	889.88 ± 11.18	98.88	1.25	−1.12	892.23 ± 44.98	99.14	5.04	−0.86
	1600	1586.54 ± 80.32	99.16	5.06	−0.84	1589.59 ± 104.43	99.35	6.57	−0.65
5-Hydroxyomeprazole	4	3.87 ± 0.16	96.68	4.05	−3.32	3.81 ± 0.09	95.33	2.29	−4.67
	80	77.38 ± 2.31	96.73	2.98	−3.27	82.00 ± 2.25	102.51	2.74	1.64
	250	248.64 ± 9.66	99.46	3.89	−0.01	252.48 ± 10.72	100.99	4.25	−0.34
	400	390.87 ± 18.05	97.72	4.62	−2.12	397.70 ± 19.96	99.42	5.02	−0.57
Omeprazole sulfone	4	3.86 ± 0.18	96.45	4.61	−3.55	3.97 ± 0.10	99.35	2.43	−0.65
	80	77.54 ± 2.54	96.93	3.27	−3.07	82.05 ± 2.81	102.56	3.42	2.56
	400	391.52 ± 17.36	97.88	4.44	−2.12	397.15 ± 19.06	99.29	4.80	−0.71
	800	788.24 ± 21.30	98.52	2.7	−1.47	795.11 ± 38.97	99.39	4.90	−0.61

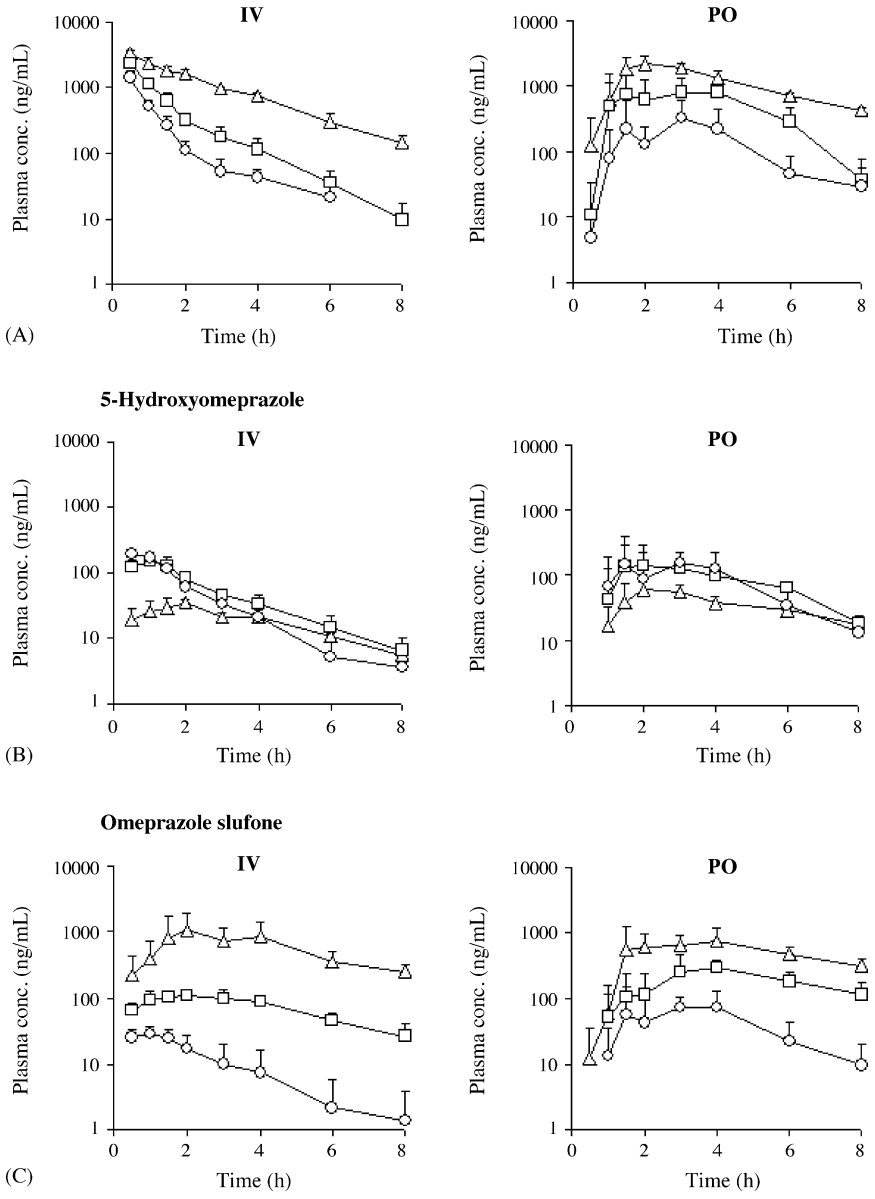

Fig. 3. Plasma concentrations-time curves (mean ± S.D.) of (A) omeprazole, (B) 5-hydroxyomeprazole, and (C) omeprazole sulfone after IV dosing (20 mg, left) or PO dosing (40 mg, right) of omeprazole in homozygous EMs (open circles), heterozygous EMs (open squares) and PMs (open triangles), respectively.

of omeprazole, 5-hydroxyomeprazole and omeprazole sulfone. Intra- and inter-day precisions were assessed by analyzing six quality control samples at each concentration on the same day and mean values of a quality control for six days, respectively. The precision determined at each concentration level should not exceed 15% of the CV except for the lower limit of quantification (LLOQ), where it should not exceed 20% of the CV [32]. These extracts underwent the same column-switching procedure. Intra- and inter-day relative standard deviations were less than 5.1 and 6.6% for omeprazole, 4.6 and 5.0% for 5-hydroxyomeprazole and 4.6 and 4.9% for omeprazole sulfone, respectively (Table 3). Accuracy was expressed as percent error (relative error) [(measured concentration − spiked concentration)/spiked concentration] × 100 (%), which precision was quantitated by calculating intra- and inter-CV values.

3.6. Stability

The stock solutions (methanol solution) of omeprazole, 5-hydroxyomeprazole and omeprazole sulfone were stable at −20 °C for at least 6 months, and spiked omeprazole, 5-hydroxyomeprazole and omeprazole sulfone in the blank plasma were stable at −20 °C for 6 months. Plasma samples for the pharmacokinetic study were stored at −20 °C and analyzed within 3 months after sampling, and then were stable at −80 °C for 12 months. Additionally, omeprazole, 5-hydroxyomeprazole, omeprazole sulfone and I.S. in extracts from plasma samples reconstituted in the mixture of 50 mM disodium hydrogen phosphate buffer (pH 9.3) and methanol were stable at room temperature for 72 h in the autosampler.

3.7. Drug concentration in human plasma

Fig. 3 shows mean plasma concentration–time curves of omeprazole and its metabolites after IV dosing (20 mg) or PO dosing (40 mg) of omeprazole to three different CYP2C19 genotype groups. The mean pharmacokinetic parameters of omeprazole and its two metabolites in three genotype groups are summarized in Table 4. In this study, plasma concentrations of 5-hydroxyomeprazole showed <10 ng/ml at 6 h after IV dosing in three genotype groups, and plasma concentrations of omeprazole sulfone in homozygous EMs also showed <10 ng/ml at 3 h after IV dosing and 6 h at PO dosing. Therefore, this method showed a more sensitive and practical method enabling monitoring of plasma concentrations of omeprazole and its two major metabolites up to 8 h after IV dosing (20 mg) and PO dosing (40 mg) of omeprazole in all volunteers because the limits of detection and quantification in previous methods was ≥10 ng/ml for 5-hydroxyomeprazole and omeprazole sulfone [22–24]. Similar to previous reports [14,28], plasma concentrations of omeprazole and omeprazole sulfone in PMs were significantly higher than that of homozygous EMs and heterozygous EMs, whereas pharmacokinetic parameters of 5-hydroxyomeprazole were opposite to that observed for omeprazole and omeprazole sulfone (Table 4). Hydroxylation index

Table 4
Pharmacokinetic parameters of omeprazole and its two primary metabolits in the three different CYP2C19 genotyping groups after an intravenous administraion (IV) of omeprazole (20 mg) and an oral administration (PO) of omeprazole (40 mg)

	Homozygous EMs	Heterozygous EMs	PMs
IV			
Omeprazole			
AUC_{0-8} (ng h/ml)	1128 ± 270	2427 ± 281*	7808 ± 634$^{***,\#\#\#}$
$t_{1/2}$ (h)	2.0 ± 0.9	1.2 ± 0.2	1.8 ± 0.3
5-Hydroxyomeprazole			
C_{max} (ng/ml)	191 ± 40	155 ± 49	37 ± 7$^{**,\#\#}$
t_{max} (h)	0.1 ± 0.0	0.6 ± 0.3	1.5 ± 0.6$^{**,\#}$
AUC_{0-8} (ng h/ml)	352 ± 70	408 ± 112	153 ± 23$^{*,\#\#}$
$t_{1/2}$ (h)	1.4 ± 0.5	1.6 ± 0.2	2.5 ± 0.3$^{*,\#}$
Hydroxylation index	3.2 ± 0.4	6.3 ± 2.0	52.3 ± 10.3$^{***,\#\#\#}$
Omeprazole sulfone			
C_{max} (ng/ml)	28 ± 8	121 ± 18	1183 ± 804$^{*,\#}$
t_{max} (h)	0.7 ± 0.3	2.3 ± 1.0	3.0 ± 1.2*
AUC_{0-8} (ng h/ml)	80 ± 52	588 ± 57	4796 ± 3323*
$t_{1/2}$ (h)	1.3 ± 0.6	2.6 ± 1.0	3.5 ± 2.5
Sulfoxidation index	17.0 ± 6.7	4.1 ± 0.1**	2.1 ± 1.1**
PO			
Omeprazole			
C_{max} (ng/ml)	523 ± 145	1396 ± 421	2288 ± 707**
t_{max} (h)	2.5 ± 0.9	2.4 ± 1.4	2.1 ± 0.6
AUC_{0-8} (ng h/ml)	1023 ± 432	3725 ± 1235	8617 ± 1739$^{***,\#\#}$
$t_{1/2}$ (h)	1.5 ± 0.5	1.0 ± 0.5	2.4 ± 0.4$^{\#}$
5-Hydroxyomeprazole			
C_{max} (ng/ml)	296 ± 129	199 ± 87	65 ± 19**
t_{max} (h)	2.8 ± 1.3	2.5 ± 1.2	2.4 ± 0.8
AUC_{0-8} (ng h/ml)	597 ± 175	608 ± 199	256 ± 67
$t_{1/2}$ (h)	1.1 ± 0.3	1.7 ± 0.2	3.6 ± 1.8
Hydroxylation index	1.8 ± 0.6	6.5 ± 2.2	34.1 ± 4.3$^{***,\#\#\#}$
Omeprazole sulfone			
C_{max} (ng/ml)	136 ± 30	319 ± 144	982 ± 541*
t_{max} (h)	2.8 ± 1.3	3.8 ± 0.5	3.4 ± 1.3
AUC_{0-8} (ng h/ml)	308 ± 75	1338 ± 625	3800 ± 1573$^{**,\#}$
$t_{1/2}$ (h)	1.3 ± 0.4	3.0 ± 0.9	3.8 ± 1.8
Sulfoxidation index	3.3 ± 0.9	2.9 ± 0.6	2.5 ± 0.8

C_{max}, peak concentration; t_{max}, time to C_{max}; AUC, area under plasma concentration–time curve; $t_{1/2}$, elimination half-life; hydroxylation index, AUC (omeprazole)/AUC (5-hydroxyomeprazole); sulfoxidation index, AUC (omeprazole)/AUC (omeprazole sulfone).

* $p<0.05$, compared with the homozygous EM group.
** $p<0.01$, compared with the homozygous EM group.
*** $p<0.001$, compared with the homozygous EM group.
$^{\#}$ $p<0.05$, compared with the heterozygous EM group.
$^{\#\#}$ $p<0.01$, compared with the heterozygous EM group.
$^{\#\#\#}$ $p<0.001$, compared with the heterozygous EM group.

after IV and PO dosing was significantly higher in PMs than in homozygous EMs and heterozygous EMs ($p < 0.001$). The relative AUC ratios of omeprazole in homozygous EMs, heterozygous EMs and PMs were 1:2.2:6.9 after IV dosing, and 1:3.6:8.4 after PO dosing, respectively. Furthermore, in our previous report [26], the relative AUC ratios of lansoprazole in three genotype groups were 1:1.3:4.2 after PO dosing. Therefore, in this result, we confirmed that omeprazole was better CYP2C19 probe than lansoprazole.

4. Conclusion

The HPLC procedure described for the simultaneous determination of omeprazole, 5-hydroxyomeprazole and omeprazole sulfone is suitable for routine analysis even though a lower pH is required for the mobile phase (eluent A, pH 7.0; eluent B, pH 6.5). Satisfactory validation data were achieved for linearity, precision and recovery. The limit of quantification obtained allows application to measuring pharmacokinetics in relation to CYP2C19 genotypes.

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